



## Quantifying the response of wheat yields to heat stress: The role of the experimental setup



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### ABSTRACT

Previous studies suggested a wide range of sensitivities of wheat yields to heat stress around anthesis. The aim of this study was to improve the understanding of the reasons of the disagreement by testing the response of wheat yield and yield components to differences in the method of heating, the temperature measurement point and soil substrate under sole heat and combined heat and drought stress around anthesis. Growth chamber experiments performed at different sites showed that increasing of the ambient air temperature at anthesis corresponding to a temperature sum of 12000 °C min above 31 °C resulted in a significant yield reduction of –24% for plants grown on sandy soil substrate but not for those grown on a soil with high soil water holding capacity. The grain yield of wheat also declined by –16% for sandy soil substrate but at a much lower level of heat stress when the temperature of the ears was increased by infrared heaters (a temperature sum of 1900 °C min above 31 °C). The yield reduction increased significantly under combined heat and drought compared to sole heat stress. Grain number significantly declined in all experiments with heat stress and combined heat and drought stress at anthesis. Single grain weight increased with heat stress around anthesis and partly compensated for lower grain numbers of pots containing a soil with high soil water holding capacity but not in experiments with sandy soil substrate. We demonstrate, based on data from previous heat stress studies, that statistical relationships between crop heat stress and yield loss become stronger when separating the data according to the soil used in the experiments. Our results suggest that the differences in the yield response to heat may be caused by additional drought stress which is difficult to avoid in heat stress experiments using sandy soil substrate. We conclude that differences in the experimental setup of heat stress experiments substantially influence the crop response to heat stress and need to be considered when using the data to calibrate crop models applied for climate change impact assessments.

### 1. Introduction

Climate is one of the most important yield determining factors explaining 30–50% of global yield variability (Frieler et al., 2017; Ray et al., 2015; Zampieri et al., 2017). The response of crop growth and yield to increasing temperatures under climate change conditions (Delworth and Knutson, 2000; Karl et al., 2015) received therefore increasing attention (Lobell et al., 2011a). For example it was estimated, that a 1 °C increase in global temperature could reduce the

global wheat yield by 4.1% to 6.4% depending on the method used for yield projection (Liu et al., 2016b).

Increase in mean temperature results mainly in a shortening of the length of the growing season by acceleration of the development rate (Asseng et al., 2015). Climate change does not only increase the mean temperature during the growth season but also intensifies the frequency of extreme heat events (Teixeira et al., 2013). There is growing experimental evidence that short episodes of very high temperature around anthesis of cereal crops can significantly reduce the grain yield

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(Eyshi Rezaei et al., 2015; Talukder et al., 2014). The major negative effect of heat stress around anthesis ( $HS_A$ ) on crop yield is that grain number per ear is reduced (Ferris et al., 1998) because of pollen abortion and sterile grains (Calderini et al., 1999; Farooq et al., 2011; Lobell et al., 2011b; Wheeler et al., 1996). In most previous studies for wheat, a threshold temperature of 31 °C was assumed for grain number reduction in the period around anthesis (Eyshi Rezaei et al., 2015) but other studies indicated 30 °C (Liu et al., 2016a) or 27 °C (Tashiro and Wardlaw, 1989) as heat stress threshold temperature in wheat. The period around anthesis is often defined as a time interval from mid of heading to end of anthesis (Ferris et al., 1998; Wheeler et al., 1996), corresponding to a period of nearly 15 days before to 5 days after anthesis (Ferris et al., 1998; Fischer et al., 1985; Ortiz-Monasterio et al., 1994; Wheeler et al., 1996). Experimental studies to evaluate the effect of heat stress on wheat yield and yield components have mainly been conducted in growth chambers (Hays et al., 2007; Narayanan et al., 2015; Prasad et al., 2011) as pot experiments, while temperature gradient tunnels (Ferris et al., 1998; Wheeler et al., 1996) and temperature free-air controlled enhancement (T-FACE) approaches (Kimball, 2005; Kimball et al., 2008) were less frequently used.

The results of previous  $HS_A$  studies performed under controlled conditions (Fig. 1 and Supplementary Table 1) are extremely diverse in terms of the response of wheat yield to different levels of heat (Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2015; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2013). For instance, grain yield loss of wheat amounted to 20% at a stress thermal time (STT) of 12000 °C min at ambient air temperature ( $T_{air}$ ) above 31 °C (Wollenweber et al., 2003) and to 95% at an almost similar stress thermal time of 14400 °C min at ambient air temperature above 31 °C (Zhang et al., 2010).

Crop models used in climate change impact assessments (Ewert et al., 2015) have been calibrated with experimental data showing a high heat sensitivity (Semenov and Shewry, 2011; Trnka et al., 2014) or lower sensitivity of wheat to heat stress (Liu et al., 2016a). To reduce corresponding uncertainties in model results, it is therefore fundamental to understand better the potential source of this inconsistency in different heat stress studies.

The sources of uncertainties in  $HS_A$  may be explained by varying experimental setup such as the temperature measurement point (ear, leaf, canopy or ambient air), method of heating (ambient air heating in growth chambers or direct heating of ears by infrared heaters), soil texture and pot size which have not been investigated systematically before. Under well-watered conditions, evapotranspiration will result in

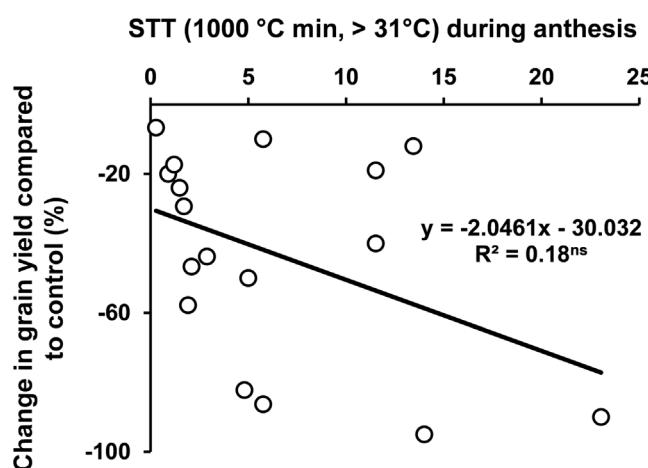


Fig. 1. The relationship between relative yield reduction and stress thermal time (STT) for 8 published studies about heat stress effects around anthesis on crop yield which provided detailed information of soil, experimental setup and applied treatments (Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2015; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2010, 2013).

a cooling of soil, vegetation surface and air inside the canopy while canopies may heat up under drought because of the reduced transpiration. Consequently, there might be considerable differences in organ temperature, canopy temperature, air temperature above the canopy and heat stress intensities calculated based on these temperatures. For example, canopy temperature was up to 7 °C higher than air temperature measured 2 m above the canopy in drought stressed rye plots, while canopy temperature was up to 6 °C lower than ambient air temperature for irrigated plots (Siebert et al., 2014). The findings of  $HS_A$  studies can also be influenced by undesirable occurrences of drought due to exacerbated impact of combined heat and drought in comparison to the sole heat effect on wheat yield (Grigorova et al., 2011; Mahrookashani et al., 2017; Wang et al., 2010). Objectives of the present study are (i) to critically compare and summarize the results of a series of independent studies with similar objectives which used different experimental setups to quantify the response of wheat yield to heat stress and combined heat and drought stress around anthesis, and (ii) to investigate whether the differences found in the sensitivity of wheat yield to the stress treatments could be associated with differences in the experimental design, in particular with respect to the temperature measurement point, heating method and the soil substrate used in the experiments.

## 2. Materials and methods

### 2.1. Experimental design and treatments setup

#### 2.1.1. Overview

A series of 6 pot experiments was carried out from 2013 to 2015 under controlled conditions at the University of Bonn (50.72 N, 7.08 E), University of Halle (51.25 N, 11.45 E) and Thünen Institute of Biodiversity Braunschweig (52.18 N, 10.26 E) to study the impact of heat stress and combined heat and drought stress on wheat yield. The experiments originally were not designed to systematically evaluate the effects of different experimental setups on crop response to heat stress around anthesis. However, experimental data were collected systematically, differences between the experiments with regard to the temperature measurement point, the heating method and the soil substrate were documented and the effect on yield response to heat and drought stress was tested. The ambient air temperature close to the wheat canopy ( $T_{air}$ ) in the growth chamber was measured in the experiments performed at Bonn and Braunschweig, while ear surface temperature ( $T_{ear}$ ) was measured in the Halle experiments. Based on these temperature measurements, heat stress was calculated as the temperature sum (°C min) above the threshold temperature of 31 °C. At the Braunschweig experiments, heating was not only applied during anthesis as in the other experiments but also at heading and after anthesis. The experiments in Halle were explicitly designed to test the effect of the heating method and temperature measurement point on the  $HS_A$  (Tables 1 and 2). A soil substrate with relatively low water holding capacity was used in the experiments at Bonn and Halle, but not in Braunschweig where the water storage capacity of the substrate was high. The plant developmental stage was determined using the BBCH scale (Lancashire et al., 1991). The experiments were performed using the winter wheat cultivar Batis or the spring wheat cultivar Ethos, which are genetically quite similar but differ in vernalization demand.

#### 2.1.2. Bonn experiments

Two pot experiments were conducted in season 2013/2014 (October–June) and 2014/2015 (November–June) in a greenhouse and growth chambers. The treatments were heat stress and combined heat and drought stress for both years in 4 replications. Plants of winter wheat (cv. Batis) were grown in pots (height 26 cm, bottom edge length 22 cm, top edge length 22 cm, 12 plants/pot) filled with a soil substrate of low water holding capacity containing 85% sand and 3% clay (Table 1). The plants were cultivated at ambient conditions until

**Table 1**

Pot size, water supply and climatic conditions in the growth chambers during heating treatments in Bonn, Halle and Braunschweig.

Experimental conditions	Bonn	Halle	Braunschweig
Pot height, bottom and top edge (cm)	26; 22; 22	16; 13; 16	20; 9; 9
Soil volume (lit)	9.0	2.4	1.2
Plants per pot	12	6	5
Soil texture	sandy	sandy loam	Mixture of peat and clay
UL (vol%)	20	38	70
LL (vol%)	5	7	15
aSWC (vol%)	18	27	55
bSWC (vol%)	5	14	30
Appointed time of watering (during the heating period)	Once daily	Once daily	Twice daily
PAR at plant height ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	500	300	500
Temperature range at heating (°C)	32–40 (air)	32–36 (ear)	36–40 (air)
Duration of heating (h per day; days)	6; 5	2; 5	15; 2–3
Relative air humidity (%)	45–65	50–60	55–65
Cultivar	Batis	Batis	Ethos

UL: Maximum soil water holding capacity in the pot, LL: Soil water content at the permanent wilting point, aSWC: Adjusted soil water content after watering, bSWC: Minimum of soil water content just before watering.

beginning of the anthesis and then moved to a growth chamber for the heat stress treatment. Before anthesis, watering was carried out by an automatic drip irrigation system. All macro and micro nutrients were supplied by using the irrigation system. The heat stress was imposed by heating the air in the growth chamber starting at anthesis (BBCH 60) and continuing for 5 days.  $T_{\text{air}}$  was gradually increased and kept between 32 °C and 40 °C for 6 h on those 5 days resulting in a stress thermal time of 12000 °C min at  $T_{\text{air}}$  above 31 °C. The temperature measurement point was located inside of the chamber close to the plants. The drought stress for the combined heat and drought treatment was initiated 10 days before heading and lasted until the end of the heat

stress period 5 days after anthesis. Soil moisture was kept close to 40% of total plant available soil water capacity (Tables 1 and 2). The relative air humidity in the growth chamber was between 45% and 65%.

### 2.1.3. Halle (Saale) experiments

The climate chamber experiments were carried out in period January–June in 2013 and 2015 and comprised three levels of heat stress and two levels of combined heat and drought stress. Plants of winter wheat (cv. Batis) were grown in pots (height 16 cm, bottom edge length 13 cm, top edge length 16 cm, 6 plants/pot) on sandy loam soil (Table 1). After vernalisation, the photon flux density was set to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at plant height. After beginning of shooting, day (16 h)/night  $T_{\text{air}}$  was set to 16 °C/12 °C and after beginning of heading to 22 °C/18 °C. The pots were watered in daily intervals and macro and micro nutrients were supplied by irrigation water. The heating was performed on 5 days (2 h/day) during anthesis by two infrared radiators (2 kW each). The ambient air temperature was elevated to 28 °C during the heating hours. The infrared radiators were arranged on two sides above the treated plants at an angle of 45 ° and at a distance of 40 cm to 60 cm to get different treatment groups of the temperature of the ears of the main tillers (32 °C to 36 °C). Related temperature sums based on  $T_{\text{ear}}$  ranged from 500 to 2600 °C min and corresponded to those reported by Ferris et al. (1998) based on  $T_{\text{air}} - T_{\text{ear}}$  was measured by an infrared thermometer (Testo 845, Testo GmbH & Co., 79849 Lenzkirch, Germany) on three ears (main tillers) of three pots representing 9 replications per treatment. For simultaneous drought, the watering in the morning was adjusted during five days before and then during 5 days at anthesis to obtain an upper value of soil water content of 30% of soil water capacity (SWC) during the heat treatment days. Thereafter, soil moisture was increased to 50% of SWC (Tables 1 and 2). Since water was supplied to reach the above-mentioned target values only once per day in the morning, the soil water content usually dropped from these maximum values to lower ones during the day, where the minimum was obtained next morning before watering. The relative air humidity was between 50% and 60% under different heating treatments.

**Table 2**

Summary of experimental setup and arrangement of sole heat and combined heat and drought stress treatments in Bonn, Halle and Braunschweig experiments.

Location	Experiment	Treatment	Start of heating (BBCH)	Heat dose (STT, °C min)	Heating method	Set-point drought intensity (SAW)	Temperature measurement point	
Bonn	E1 (2014)	Control	–	–	–	–	Air	
		Heat	60	12000	Growth chamber	–		
	E2 (2015)	Heat + Drought	60	12000	–	40		
		Control	–	–	–	–		
		Heat	60	12000	Growth chamber	–		
	E1 (2013)	Heat + Drought	60	12000	–	40		
Halle		Control	–	–	–	–	Ear	
		Heat	60	1400	Infrared heater	–		
E2 (2015)	Heat + Drought	60	800	–	30			
	Control	–	–	–	–			
	Heat1	60	500	Infrared heater	–			
	Heat2	60	1900	–	–			
Braunschweig	E1 (2014)	Heat + Drought	60	2600	–	30	Air	
		Control	–	–	–	–		
		Heat1	50	13000	Growth chamber	–		
		Heat2	60	8000	–	–		
	E2 (2014)	Heat3	68	10000	–	–	Air	
		Control	–	–	–	–		
		Heat1	50	12000	Growth chamber	–		
		Heat2	60	12000	–	–		
		Heat3	68	16000	–	–		

SAW: Soil available water.

### 2.1.4. Braunschweig experiments

Two pot experiments were conducted under controlled conditions from December 2013 until May 2014. The treatments were roughly similar levels of heat stress at three different phenological stages including heading (BBCH 50), anthesis (BBCH 60) and after anthesis (BBCH 68) with 4 replications. Plants of spring wheat (cv. Ethos) were grown in pots (height 20 cm, bottom edge length 9 cm, top edge length 9 cm, 5 plants/pot) on a soil substrate containing a mixture of peat and clay with high water holding capacity (70 vol%) (Table 1). All macro and micro nutrients were supplied to the soil before the start of the experiments. The plants were grown up in a greenhouse and then moved at heading, anthesis and after anthesis to a growth chamber for heat stress treatments. All of the side tillers were cut during the early growth period. During the heating treatments pots were watered at the start and end of the light period to a constant weight. The heat stress treatments were imposed by increasing  $T_{air}$  and adjusting air humidity with a humidifier in the growth chambers.  $T_{air}$  and relative humidity inside the growth chamber were measured with a ventilated thermistor located near the plants at the height of the flag leaves, and data were continuously recorded with a logger (Manderscheid et al., 2016). The heat treatments (15 h per day) were as follows at BBCH 50, E1: 36 °C × 3 days, E2: 36 °C × 2 days; at BBCH 65, E1: 36 °C × 2 days, E2: 38 °C × 2 days; at BBCH 68, E1: 38 °C × 2 days, E2: 40 °C × 2 days (Table 2). The relative air humidity inside of the growth chamber was between 55% and 65% under different heating treatments.

### 2.2. Plant measurements

Grain yield, grain number and single grain weight were obtained from main stems when plants reached maturity. The harvested grains were dried for 48 h at 100 °C to measure the dry weight. Images of plant surface temperature during the heating treatment in the growth chamber were taken using a thermographic camera (Fluke Ti32, Fluke Cooperation, USA) at Braunschweig climate chambers.

### 2.3. Data analysis

The design of all experiments was a completely randomized. A one-way ANOVA was performed to test the significance of the applied heat and heat + drought combinations on wheat yield and yield components. The Fisher's protected least significant difference (LSD) test was employed to identify the mean differences between the treatments. The letters *a*, *b* and *c* represented significant difference in mean values. The relationships between study variables were tested using linear regression. The "agricolae" package embedded in R language (R Development Core Team 2012) was used to perform the statistical tests.

## 3. Results

### 3.1. Sole effects of heat stress on yield and yield components

#### 3.1.1. Grain yield

Results of the experiments in Bonn and Halle showed a significant decline in grain yield of wheat (−24% – −16%) by imposing STT of 12000 °C min ( $T_{air} > 31$  °C) and 1900 °C min ( $T_{ear} > 31$  °C) at anthesis stage, respectively (Fig. 2a). A low heat stress (STT of 500 °C min ( $T_{air} > 31$  °C)) did not result in significant differences of wheat yield in Halle experiments compared with the control. Application of similar heat intensity (STT of 8000 – 16000 °C min ( $T_{air} > 31$  °C)) at different phenological stages from heading to end of anthesis did not significantly influence the grain yield of wheat in Braunschweig experiments (Fig. 2a).

#### 3.1.2. Grain number

Imposing of heat at anthesis stage significantly reduced the grain number in all experiments (Fig. 2b). However, the magnitude of

reduction in grain number was different across the experiments. Application of heat stress during the anthesis stage reduced the grain number by −11% to −22% across the experimental sites, however, heating at heading stage reduced the grain number by −38% at Braunschweig (Fig. 2b).

#### 3.1.3. Single grain weight

The single grain weight showed a small non-significant decline or no change under different heat stress intensities at anthesis and after anthesis stages in all experiments (Fig. 2c). However, application of heat stress at heading stage in Braunschweig increased the single grain weight by +35% (Fig. 2c).

### 3.2. Combined effects of heat and drought stress on yield and yield components

#### 3.2.1. Grain yield

Combined heat and drought at anthesis stage caused a remarkable yield decline (−85%) compared to sole heat treatment in Bonn experiments (Fig. 2a). The magnitude of yield decline under combined heat and drought treatments was slightly smaller (−50%) but still significant in Halle experiments (Fig. 2a). Even a small amount of heat stress at anthesis (STT of 800 °C min ( $T_{ear} > 31$  °C)) caused a significant yield decline in combination with drought in Halle experiments (Fig. 2a).

#### 3.2.2. Grain number

The grain number was strongly reduced under combined heat and drought stress at anthesis by −87% – −80% in Bonn and Halle experiments, respectively (Fig. 2b). The variability of grain number across replications was also increased under combined heat and drought stress compared to control and sole heat treatments (Fig. 2b).

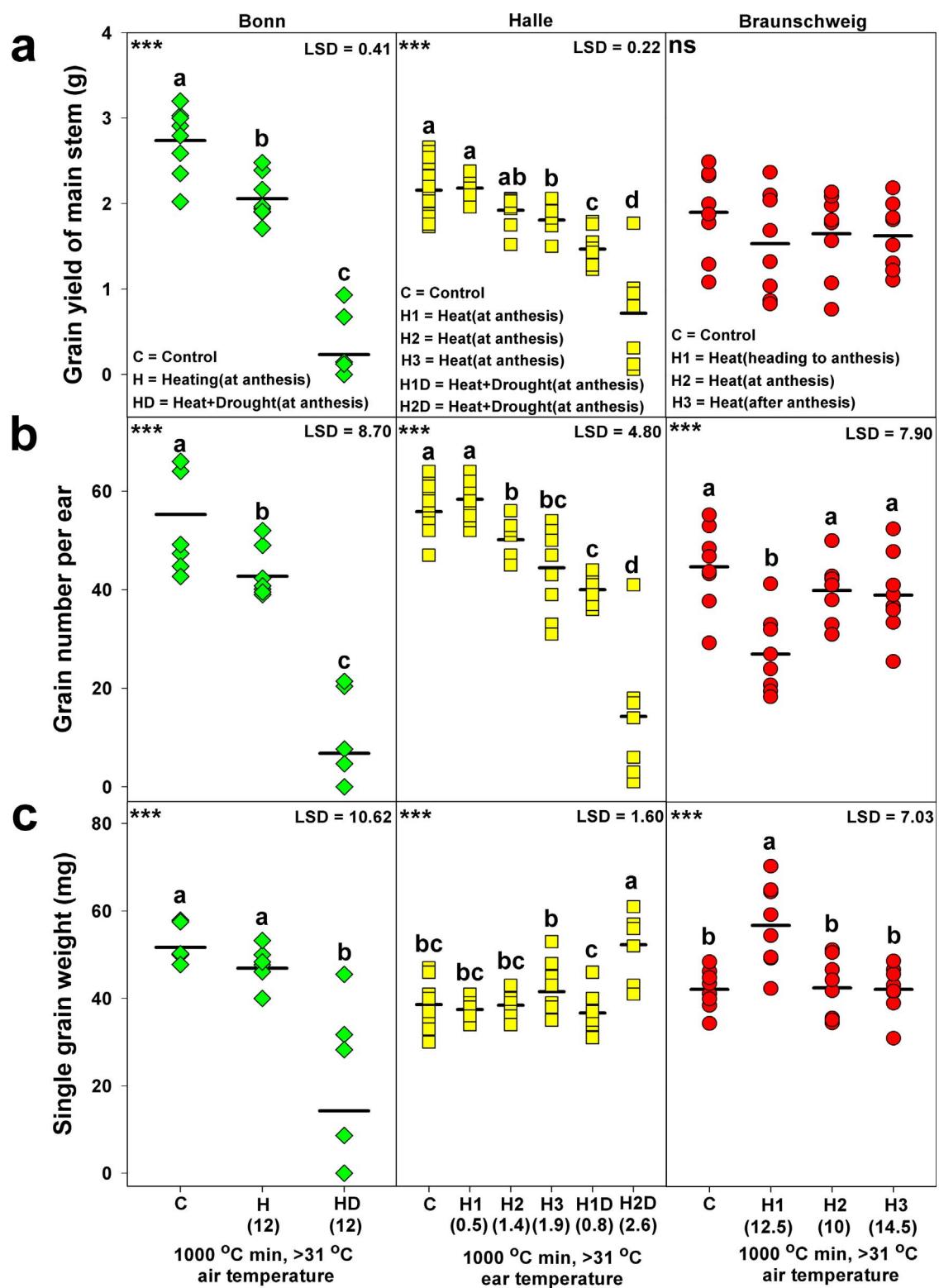
#### 3.2.3. Single grain weight

The single grain weight of wheat showed a diverse response to combined heat and drought stress in Bonn and Halle experiments (Fig. 2c). It increased by +30% under combined heat (STT of 2600 °C min ( $T_{ear} > 31$  °C)) and drought stress in Halle experiments (Fig. 2c). In contrast, the combined heat (STT of 12000 °C min ( $T_{air} > 31$  °C)) and drought stress reduced the single grain weight by −80% in Bonn experiments (Fig. 2c).

### 3.3. The relationships between yield and yield components under sole heat and combined heat and drought stress

As expected, there was a positive relationship between grain yield and grain number in all experiments. This relationship was strong and significant for the Bonn ( $R^2 = 0.84$ –0.93) and Halle ( $R^2 = 0.72$ –0.93) experiments under sole heat and combined heat and drought at anthesis (Figs. 3a, b), and less strong ( $R^2 = 0.51$ ) but still significant under heat stress in Braunschweig experiments (Fig. 3c). On the other hand, there was no significant relationship between grain yield and single grain weight ( $R^2 = 0.004$ –0.19) under sole heat stress in all experiments (Fig. 4). Combined heat and drought stress resulted in a strong significant positive relationship between grain yield and single grain weight for Bonn ( $R^2 = 0.82$ ) and Halle ( $R^2 = 0.25$ ) experiments (Figs. 4a, b).

Negative non-significant and significant relationships between grain number and single grain weight were found under heat stress at anthesis stage in Bonn ( $R^2 = 0.09$ ) and Halle ( $R^2 = 0.59$ ) experiments, respectively (Figs. 5a, b). However, heat stress at anthesis stage caused a non-significant positive relationship ( $R^2 = 0.11$ ) between grain number and single grain weight in Braunschweig experiments (Fig. 5c). Relationships between grain number and single grain weight under heat stress before and after anthesis (Fig. 5c) were all non-significant. The combined heat and drought stress at anthesis resulted in a positive



**Fig. 2.** The observed grain yield (a), grain number (b) and single grain weight (c) of wheat under control, different levels of heating and combined heat and drought stress in Bonn, Halle and Braunschweig experiments. Each point represents one replication; the black line indicates the mean value. ns = non-significant trend and \*\*\* = significance at 0.1% probability level, respectively. Differences between treatments were obtained using Fisher's Least Significant Difference (LSD) test. Different letters indicate statistically significant difference ( $P \ll 0.05$ ) between treatments. C = control, H = sole heat, and HD = combined heat and drought.

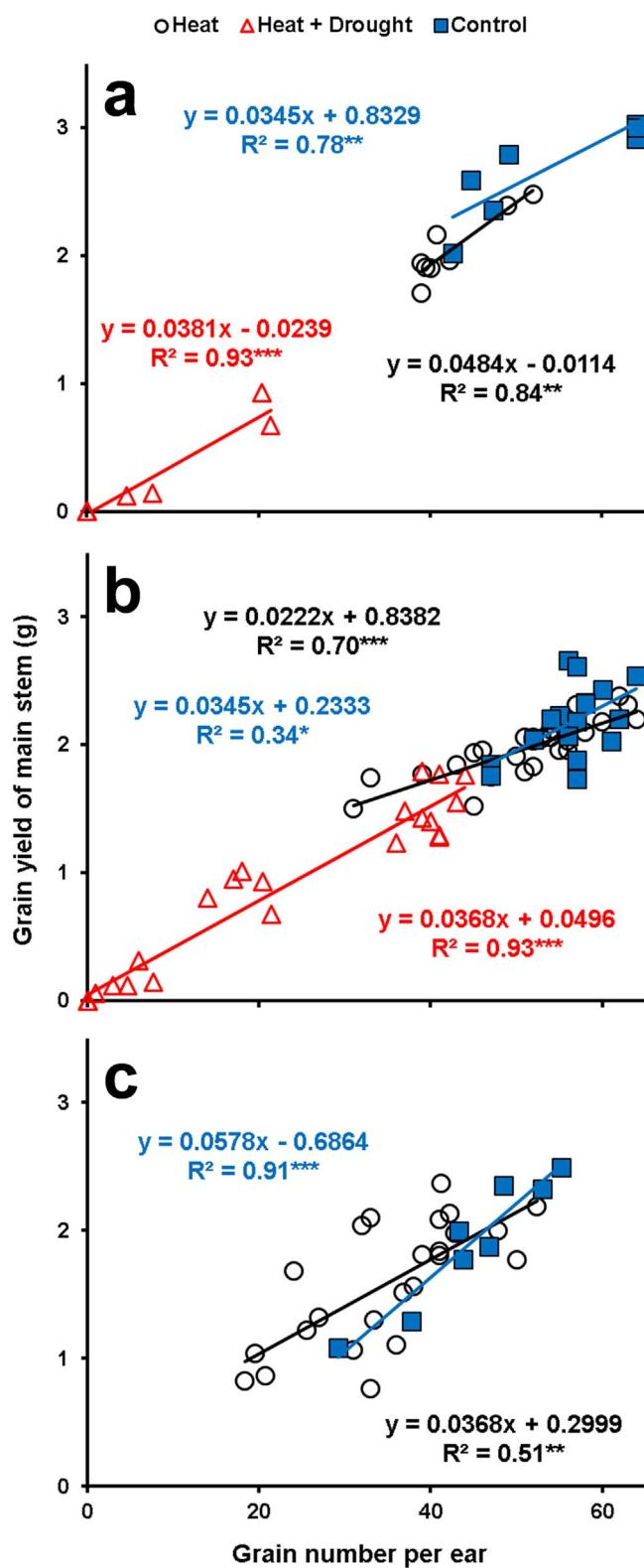


Fig. 3. The relationship between grain yield and grain number under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. \*\* and \*\*\* = significance at 1 and 0.1% probability levels, respectively.

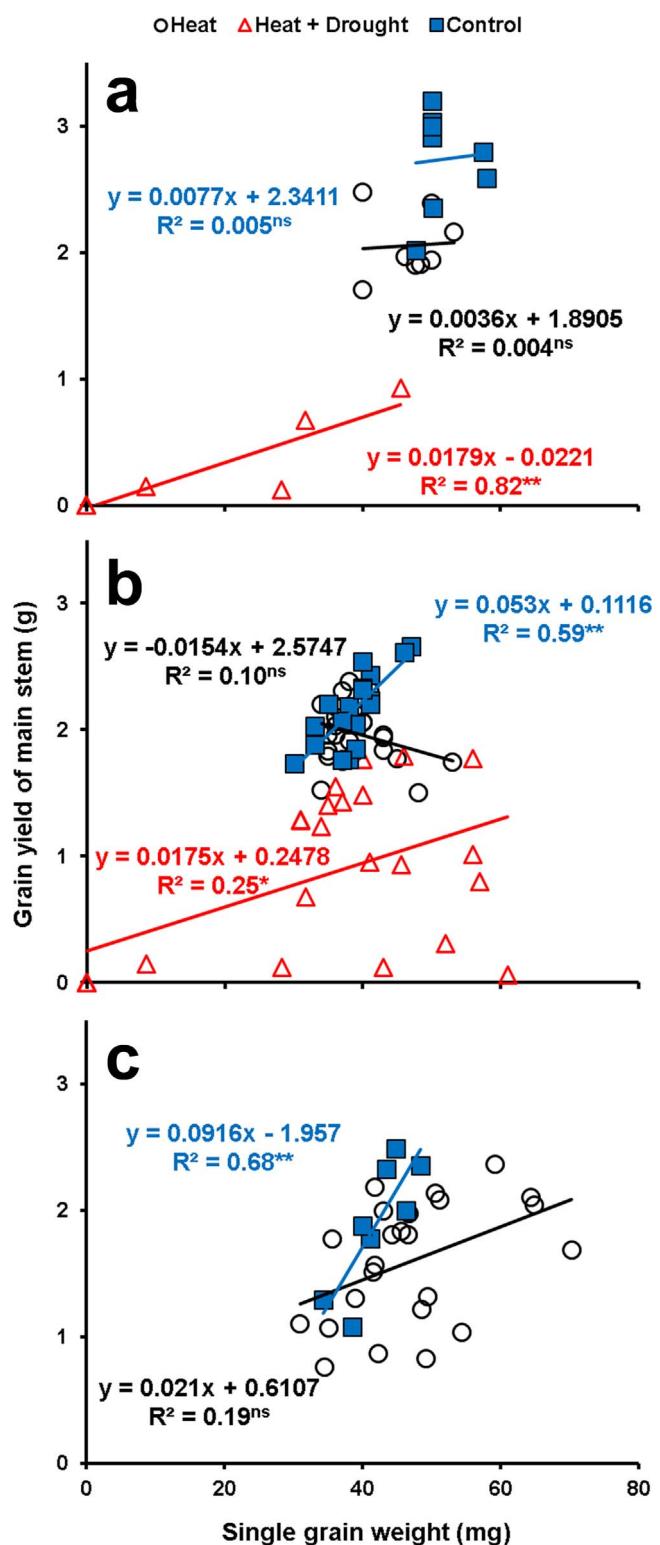


Fig. 4. The relationship between grain yield and single grain weight under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. ns = non-significant trend, \* and \*\* = significance at 5 and 1% probability levels, respectively.

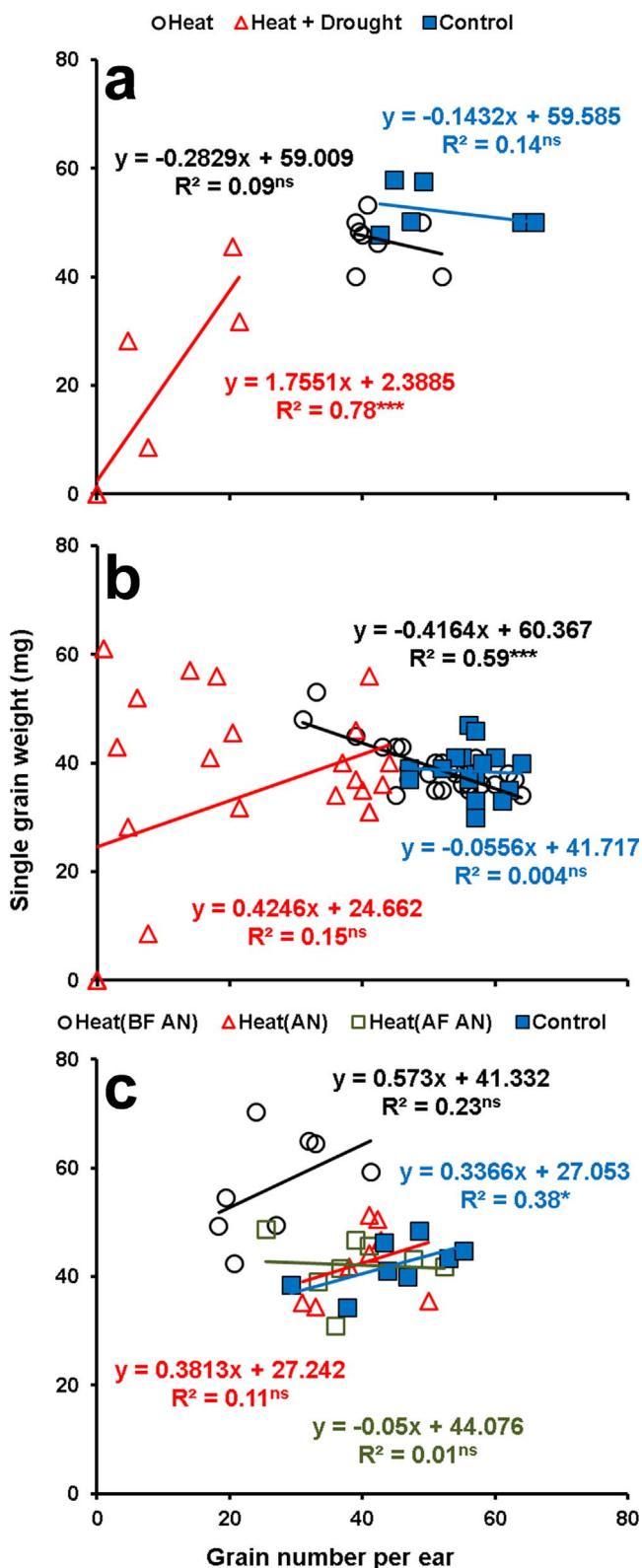


Fig. 5. The relationship between grain number and single grain weight under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. ns = non-significant trend and \*\*\* = significance at 0.1% probability level. BF AN = before anthesis, AN = anthesis and AF AN = after anthesis.

relationship between grain number and single grain weight at both Bonn ( $R^2 = 0.78$ ) and Halle ( $R^2 = 0.15$ ) experiments (Figs. 5a, b).

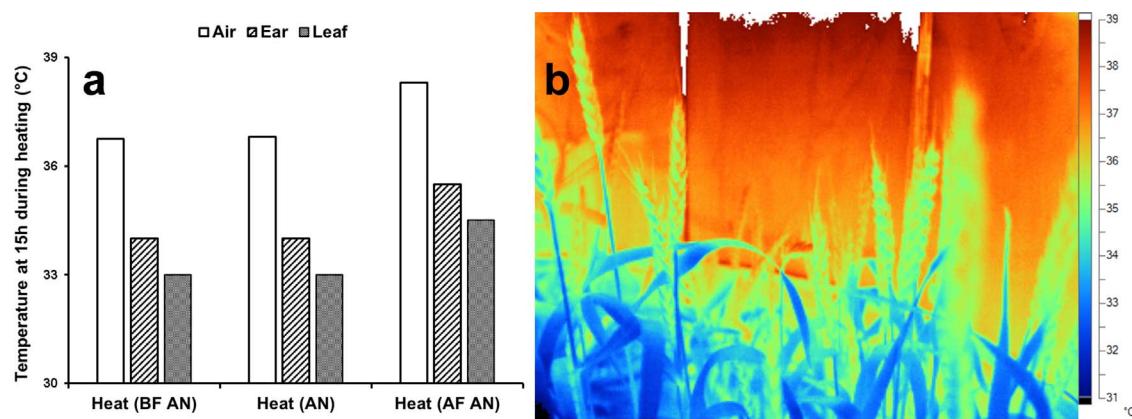
#### 4. Discussion

##### 4.1. Heat stress experiments and temperature measurement point and method

Our results show that it is essential to consider differences in the temperature measurement point and in the method used to measure temperature when interpreting the response of crops to heat stress. The relative grain yield reduction was almost similar for stress thermal times of 12000 °C min or 1900 °C min when measuring the ambient air of the growth chamber and the tissue temperature of the ear, respectively (Fig. 2a). This could be related to substantial differences between tissue, canopy and ambient air temperature at different conditions (Siebert et al., 2014) impacted by soil water status and crop transpiration rate (Jackson et al., 1981; Van Oort et al., 2014).

Our temperature measurements under heat stress in Braunschweig experiments revealed differences of 4–5 °C between ambient air temperature and leaf surface temperature and 3 °C between ambient air temperature and ear surface temperature (Fig. 6). The temperature of air, ear and leaf surface were 37.3 °C, 34.5 °C and 33.5 °C, respectively (Fig. 6). However, considering a 3 °C difference between air and ear surface temperature according to the data from the Braunschweig experiments in the calculation of STT for the Halle and Bonn experiments could only partly remove the differences in the sensitivities of wheat yields to heat. This implies that other factors than only the temperature measurement point (see next section) might be responsible for the different findings in the three experiments. We also want to highlight that the air temperature also depended on the distance of the measurement point to the plant tissue with higher air temperature at larger distance (Fig. 6b). In any case, stress thermal time and the corresponding sensitivity of wheat yields to it depends highly on the temperature measurement point and the method used to measure temperature (infrared sensor versus thermometer). Unfortunately, we did not measure air, canopy and tissue temperatures simultaneously in all experiments. However, it may be assumed that the tissue temperature could have been higher under combined heat and drought stress, such that this could be one of the reasons for the larger decline in grain yield under that treatment. It was shown before that stomatal closure under combined heat and drought stress resulted in significantly higher leaf temperature and decline in photosynthesis rate compared to effects of individual heat and drought (Mittler, 2006).

Under field conditions and for regional assessments another uncertainty is introduced by the fact that temperature measurements in the canopy or tissue temperature data are often not available. Instead, usually air temperature measurements from weather stations are used (Lobell et al., 2011a; Lobell and Asseng, 2017) although air temperature at the weather station is a poor indicator of canopy temperature (Siebert et al., 2017, 2014; Webber et al., 2017). However, the difference between air and canopy temperature observed in a growth chamber may not be appropriate to represent the relationships between air and canopy temperatures in the field because of the effects of air turbulence (wind speed), diverse soil water conditions, radiation, and energy transfer between air and canopy. For instance, the canopy temperature of groundnut and rice were about 4 °C cooler than air temperature under well-watered conditions (Jagadish et al., 2007; Prasad et al., 1999). The occurrence of combined heat and drought stress can reverse the relationship between air and canopy temperature. The leaf temperature was 4 °C higher in a combined heat and drought treatment in comparison to sole heat stress under well-watered conditions (Rizhsky et al., 2002).



**Fig. 6.** The average temperature (a) of air, leaf and ear of wheat plants during the heating treatment and the example of thermal picture (b) during the heating period in Braunschweig experiments. BF AN = before anthesis, AN = anthesis and AF AN = after anthesis.

#### 4.2. Impact of the heating method on heat stress sensitivity

Our results indicate that the heating method could be another source of uncertainty in HS<sub>A</sub> experiments. Heating of the ears directly by infrared heaters as in the Halle experiments could have led to the lesser amount of heat (STT of 1900 °C min ( $T_{\text{ear}} > 31^{\circ}\text{C}$ )) required to reduce the grain number and in consequence yield (Fig. 2), even in case of 3–5 °C temperature difference between tissue and air temperature. In contrast, whole plants will need much more heating in growth chamber experiments to eliminate the transpiration cooling effect under high temperature (Crawford et al., 2012). Another side-effect of heating of the whole plants by elevating the air temperature in growth chambers may result from the fact that also the roots will be heated up from different sides, which is not the case under field conditions. The root growth and functioning is more sensitive to heat stress than the shoot (Heckathorn et al., 2013) and can be strongly influenced by heat stress (Huang et al., 2012). However, own investigations in Braunschweig with only pot heating up to about 36 °C did not affect grain yield of Batis or Ethos (data not shown). Moreover, the quite small effect on grain yield of heating after anthesis when roots are still functioning implies that roots might not be impaired by these high temperatures.

Using infrared heaters was claimed to be the best method to reproduce natural heating effects because of minimizing the canopy disturbance under well-watered conditions (Kimball, 2011). One possible reason for higher heat response in Halle as compared to Bonn and Braunschweig may be related to the difference in leaf vapour pressure deficit (LVPD). The air temperature was not increased in Halle experiments, thus vapour partial pressure was much lower in Halle than in Bonn and Braunschweig. It seems possible that LVPD in Halle was extremely high causing a decrease of stomatal conductance and thus a decrease of photosynthesis. However, further studies and accurate measurements of LVPD are required to confirm this hypothesis. On the other hand, it may be argued that this approach may not be a suitable mimic of temperature effects related to climate change because of the strong modification of the vapour pressure gradient from leaf to atmosphere under heat and under combined heat and drought conditions and the general change in relationships between air and canopy temperature (Amthor et al., 2010; Aronson and McNulty, 2009).

#### 4.3. Impact of the soil substrate on heat stress sensitivity

We found a different yield response at similar intensity of heat stress (STT of 12000 °C min ( $T_{\text{air}} > 3^{\circ}\text{C}$ )) and the method of heating (growth chamber) when comparing the results between the Bonn and Braunschweig experiments. There was a significant yield reduction in Bonn experiments but not in the Braunschweig experiments (Fig. 2a). In addition, the significant reduction in grain number under heat stress

around anthesis in the Braunschweig experiments was compensated by increase in single grain weight (Fig. 2b and c). This compensatory effect was not observed in the Bonn experiments (Fig. 2b and c). The ultimate grain weight of wheat is determined by the rate and duration of the grain filling period (Liu et al., 2014). There is a negative relationship between grain number and individual grain weight of wheat at non-stress conditions (Dreicer et al., 2009). However, the relationship between grain number and single grain weight after imposing heat stress around anthesis varied across the studies. The reduced number of grains due to heat stress at anthesis did not consequently increase the single grain weight (Wheeler et al., 1996), likely due to heat stress damage of photosynthetic apparatus (Narayanan et al., 2016; Wollenweber et al., 2003). However, other studies showed that the single grain weight significantly increased if grain number was reduced under heat stress conditions (Stone and Nicolas, 1995). In the present experiments at Braunschweig flag leaf senescence as measured by chlorophyll content was not accelerated under the heat treatments indicating that photosynthesis was unaffected.

Our results suggest that the discrepancy in those two experiments may be caused by differences of the soil substrates. The HS<sub>A</sub> experiments in Braunschweig, were performed using a soil with very high soil water capacity (55 vol%), while the soil water capacity was reasonably low (18 vol%) in Bonn experiments. The tillers which are the potential source of transpiration to reduce the available water in a pot for each plant were cut off in Braunschweig experiments but not in the Bonn and Halle experiments. The daily maximum available water per plant during the heat experiment just after watering was ~170 ml in Braunschweig experiments and ~75 ml in Bonn experiments. Differences in the setup of the experiments with respect to pot size, plant density and water holding capacity of the soil will affect the competition for water and nutrients within a pot and thus also the crop response to heat and combined heat and drought stress. Therefore, more systematic analyses under controlled conditions are required to confirm the results of the current study.

Soil water status and transpiration rate play a key role in control of the temperature of crops under heat stress (Reynolds et al., 1994). The use of sandy soil in the Bonn and Halle experiments could have resulted into some amount of unintended drought stress as shown for the Halle experiments (Fig. S1) and thus could have limited the transpiration (Jones and Tardieu, 1998; Passioura et al., 2006). Possibly, a mild drought might have been induced by the high temperature and high plant water requirements during the heating period at Bonn. An additional increase of the ear and leaf temperature could then have resulted in an additional reduction of the grain yield due to the direct effects of drought stress on grain number and yield (Rajala et al., 2009). On the other hand, this explanation will not apply for the Halle experiments, because the evapotranspiration for the heat stress treatments was not

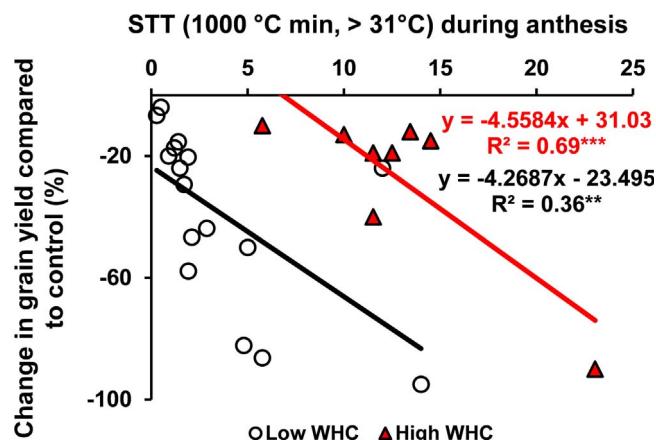


Fig. 7. The relationship between relative yield reduction and stress thermal time (STT) for 8 published studies about heat stress effects around anthesis on crop yield which provided detailed information of soil, experimental setup and applied treatments (Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2015; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2010, 2013) and results of Bonn, Braunschweig and Halle experiments. The studies were divided based on the soil substrate of pots to substrate with high and low water holding capacity (WHC). \*\* and \*\*\* = significance at 1 and 0.1% probability levels, respectively.

remarkably increased in comparison to the control and drought treatments without heating (Fig. S2). Therefore, in the Halle experiment the accelerating effect of drought on the response to heat stress might be attributed to a direct impairment of fertility (pollen growth) by drought during anthesis. Finally, the high proportion of peat of the soil used in Braunschweig experiments probably avoided the effect of unintended drought that might have been led to yield reduction by an extra heat effect as assumed above for the Bonn study as well by a direct drought effect on fertility as supposed for the Halle analysis.

Based on these considerations, we divided the studies that we used for meta-analysis in the introduction (Fig. 1) plus the results of our series of experiments into studies which were performed by using soil substrate with either high or low water holding capacities. There was a significant relationship between the relative reduction in grain yield and STT  $> 31^\circ\text{C}$  after dividing the studies to low ( $R^2 = 0.36$ ) and high ( $R^2 = 0.69$ ) water holding capacity of soil substrates of pot experiments (Fig. 7) while the relationship was not significant when analyzing the data in combination (Fig. 1). Differences in sensitivity of specific wheat cultivars are well documented and some of the variability presented in Fig. 7 could be caused by cultivar differences. In the present study cultivars of wheat with a similar genetic background have been used, which do not strongly differ in their response of yield and yield components to STT as indicated by the results obtained in Bonn and Braunschweig in the sole heat treatments. Moreover, experimental data from two Chinese wheat cultivars recently published in Liu et al. (2016a, 2016b) indicated that 50% grain yield loss due to heat around anthesis occurred at a stress thermal time of about 40000–50000 °C min ( $\text{Tair} > 30^\circ\text{C}$ ), which corresponds to the results for our cultivar if the data are linearly extrapolated.

Based on our results the simultaneous occurrence of drought stress under high temperature (as combined heat and drought treatment) may amplify the heat effect by reducing the transpiration and photosynthesis due to stomatal closure (Nankishore and Farrell, 2016). Such amplifying effect of combined heat and drought was also observed for other crops including groundnut, maize and cotton (Cairns et al., 2013; Dabbert and Gore, 2014; Hamidou et al., 2013). Additionally, a direct impairment of pollen growth that may occur under drought (Barnabas et al., 2007) may overlay and strengthen the effect of heat stress. This could be the main reasons of the higher yield reduction in combined heat and drought stress in Bonn and Halle experiments. We found an increase in single grain weight under combined heat and drought

treatment by reducing of grain number in the Halle H2D experiment but this compensatory effect was not observed in Bonn experiments. The different response could be related to longer duration of drought stress in Bonn experiments (10 days before anthesis) in comparison to Halle experiments (4 days before anthesis).

We also suggest that because of the predominant mechanism of interaction of heat and rising  $\text{CO}_2$  concentration a precise analysis of the sole heat effect on grain yield is necessary. If grain yield will be affected already at small levels of heat stress, then the small increase of tissue temperature due to the reduction of transpiration under elevated  $\text{CO}_2$  would intensify the effect of high air temperature and there would be a positive interaction of heat and  $\text{CO}_2$  as observed in studies with rice and sorghum (Jagadish et al., 2014). If grain yield is only impacted at high levels of heat stress, detrimental effects of heat only seems rather unlikely for the moderate climate zone of Europe even under future climate change. Such high levels of STT of the plant tissue can be expected only under restriction of transpiration by low soil moisture due to lack of rainfall. However, plants grown under elevated  $\text{CO}_2$  use less water and thus soil drying is delayed. Consequently, it is conceivable that rising  $\text{CO}_2$  concentration can also mitigate the effect of heat especially when the high tissue temperature results from restricted transpiration cooling due to drought.

## 5. Conclusion

The magnitude of heat effect on grain yield of winter wheat can be substantially affected by the experimental setup including the temperature measurement point, the method of heating, and the soil substrate used. Combined heat and drought reinforced the negative effect of high temperature on crop yield and yield components and in this way reduced the grain yield significantly even under very moderate intensity of heat. Therefore, it is fundamental to understand the source of uncertainties in such experiments in order to make proper use of the data and results obtained. Crop modellers should carefully select experimental data to be used for model calibration and testing depending on the process representation in the specific model. Data obtained from experiments differing in heating method, temperature measurement and soil substrate should not be merged but be analysed separately. The findings of our experiments imply that the observations reported in some previous studies on  $\text{HS}_A$  did not only result from heat stress impact but also from a combination of heat and drought. Climate change impact assessments performed using crop models indicated that heat will be a major threat to wheat yield under climate change. However, we suggest that many of those models have been calibrated based on studies that included a combination of heat and unintended drought. Thus, threat to wheat yield by future heat periods might have been overestimated at least for some regions such as central Europe. A more typical scenario in such regions will be peak heat phases occurring during drought periods rather than heat stress under conditions with sufficient water availability. Our results show that this combination, in particular for soils with low water holding capacity, may lead to much more dramatic yield losses than drought or heat on its own.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the

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